Section II (Remarks)

Amendments to the Specification

In response to the objection to the specification at page 10, line 25 and page 14, lines 3 and 5 as being inconsistent with the disclosure at page 6, lines 10-13, the specification has been amended herein to properly set out the units of the particle size as μm, consistent with the units set forth at page 6, lines 10-13, and consistent with the text of the international patent application (see discussion, *infra*)..

In response to the objection to the specification at page 18, line 16 to page 19, line 15, in which the parentheticals following ratios of ingredients lack the symbols shown in the drawings, the specification has been amended at page 18, lines 25-26 and page 19, line 15 to parenthetically set out the symbols shown in the drawings, as well as in the text of the international patent application (copies of the corresponding pages of which are contained in Appendix A hereof).[1]

No new matter (35 USC 132) has been added by such corrections of the specification.

Amendments to the Claims

The withdrawn claims 1-10, 12-15, 21 and 23-28 have been amended for consistency with the product claims 16-20 and 29-31 under examination, to facilitate rejoinder of the method claims under the provisions of MPEP 821.04.

Claims 11 and 22, reciting that the polyanionic salt comprises sodium tripolyphosphate, have been cancelled.

[1] In the Preliminary Amendment filed December 20, 2005 at the inception of national phase proceedings, the specification was amended to state that "The disclosures of said International Patent Application and Spanish Patent Application are hereby incorporated herein by reference, in their respective entireties, for all purposes."

Claims 1, 4, 12, 13, 16, 17, 26, 29 and 30 have been amended to recite "sodium tripolyphosphate" in place of "polyanionic salt" as formerly recited in such claims.

More specifically, previously withdrawn claims 1 and 26 have been amended herein for consistency with the product claims under examination, to facilitate rejoinder of the method claims under the provisions of MPEP 821.04, by amendment of claim 1 to recite that "the nanoparticles have no covalent bonds between the hyaluronic acid salt, cationic polymer, sodium tripolyphosphate and active ingredient," and by amendment of claim 26 to recite that "the nanoparticles have no covalent bonds between the hyaluronic acid salt, cationic polymer, and sodium tripolyphosphate."

Such amendment finds support in the product claims, e.g., claim 16, which recites that the "nanoparticles have no covalent bonds between the hyaluronic acid salt, cationic polymer, sodium tripolyphosphate and active ingredient," and claim 17, which recites nanoparticles "comprising a hyaluronic acid salt, a cationic polymer, sodium tripolyphosphate and the active ingredient as components, without covalent bonds between the components."

Claims 1, 16, 17 and 26 have been amended to recite that the nanoparticles are "characterized by a stability of at least one month at ambient temperature storage." Such recital is consistent with the disclosure in the application, at page 18, line 16 to page 19, line 1 ("Hyaluronic acid nanoparticles in the form of sodium salt, chitosan as cationic polymer and sodium triphosphate as crosslinking agent, were prepared … [p]article size and surface charge measurements were made, during one month, with the aim of obtaining information on the system evolution with time…[t]he results presented in figures 4 and 5 showed the little variability of the parameters, size and zeta potential, during the storage").

The independent method claims thus contain the limitations of the product claims, as requisite for rejoinder under MPEP 821.04.

New claims 32 and 33 have been added herein, to claim specific aspects disclosed in the application. Claim 32 depends from claim 16, and recites the nanoparticles as storage stable in particle size and zeta potential for at least one month. Claim 33 depends from claim 17 and correspondingly recites the nanoparticles as storage stable in particle size and zeta potential for at least one month. The newly added claims 32 and 33 are consistent with the above-discussed disclosure at page 18, line 16 to page

19, line 1 of the application ("[p]article size and surface charge measurements were made, during one month, with the aim of obtaining information on the system evolution with time …[t]he results presented in figures 4 and 5 showed the little variability of the parameters, size and zeta potential, during the storage").

Claims 29 and 30 have been amended to recite "hyaluronic acid salt" rather than "hyaluronic acid," thereby rendering such dependent claims consistent with the antecedent basis in claim 17, from which claims 29 and 30 directly depend.

No new matter (35 USC 132) has been introduced in the application by the foregoing claim amendments/additions.

September 2, 2009 Interview with Examiner Nissa M. Westerberg

Appreciation is expressed to Examiner Nissa M. Westerberg for the courtesy extended in granting a telephonic interview to the undersigned attorney on September 2, 2009. The substance of the interview is set forth in the September 8, 2009 Interview Summary issued by the Examiner.

As therein stated, the cited Prokop and Calias references were discussed, and the examiner maintained the 35 USC112, second paragraph grounds of rejection, based on the contention that an essential step had been omitted from the claims for the production of nanoparticles, and the corresponding contention that the described procedure would not result in the formation of nanoparticles. The evidence that would be useful in demonstrating the unexpected results for the full scope of the claims was discussed, and such evidence has been provided in this response by the Declaration under 37 CFR 1.132 of Dr. Ana Isabel Vila Pena submitted concurrently herewith, and discussed more fully hereinafter.

Rejection of Claims for Double Patenting

In the June 10, 2009 Office Action, claims 16-20, 22 and 31 were provisionally rejected on the ground of nonstatutory obviousness-type double patenting over claims 1-12 of copending Application No. 12/301,835.

In view of the provisional character of this rejection, applicants respectfully request that the double patenting issue be deferred, pending the identification of allowable claims by the examiner in this application.

Rejection of Claims Under 35 U.S.C.§112 and Traversal Thereof

In the June 10, 2009 Office Action, claims 16-20, 22 and 29-31 were rejected under 35 U.S.C. §112, second paragraph as being incomplete for omitting essential elements, identified in the Office Action as "a step such as the formation of an emulsion, dropwise addition of one solution to the other solution or an equivalent step that results in the formation of nanoparticles" (Office Action, page 5, lines 4-6).

Such rejection is traversed. (It is noted that claim 22 has been cancelled, thereby rendering the rejection moot in respect of such claim.)

Applicants point out that the nanoparticles of their claimed invention are spontaneously formed upon stir-mixing of the respective solutions, as recited in claim 16 (see step (d) therein, reciting "stir-mixing the solutions resulting from steps (b) and (c), spontaneously obtaining the nanoparticles"), **without any emulsion formation or dropwise addition of one solution to the other being required**.

Accordingly, **emulsion formation or dropwise addition steps are not essential elements of the process for preparing the nanoparticles of applicants' invention**.

Although it is clear and definite from the disclosure of applicants' specification that nanoparticles can be spontaneously formed by mere stir-mixing of (1) an aqueous solution of a hyaluronic acid salt to which sodium tripolyphosphate has been added, and (2) an aqueous solution of a cationic polymer, which should be conclusive of the issue, applicants have provided further empirical evidence that simple stir-mixing of such aqueous solutions will spontaneously form nanoparticles.

Such evidence is enclosed with this response, in the form of an accompanying Declaration of Ana Isabel Vila Pena submitted under the provisions of 37 CFR 1.132. In such Declaration, Dr. Pena states that she is an employee of ADVANCED IN VITRO CELL TECHNOLOGIES, S.L., of

Barcelona, Spain (hereafter referred to as "AIVCT"), having a PhD degree in chemistry, and working in the laboratory of such company.

Dr. Pena further states in her Declaration that she has been requested to conduct experimental work and to provide testimonial evidence in support of the present patent application (hereafter referred to as the "Application"), which has been assigned to AIVCT. Dr. Pena's Declaration sets out the claims 16, 17, 32 and 33 of the Application:

> **16.** Nanoparticles with a diameter less than 1μm, characterized by a stability of at least one month at ambient temperature storage, for the administration of an active ingredient, which are obtained by a method comprising (a) preparing an aqueous solution of a hyaluronic acid salt, (b) preparing an aqueous solution of a cationic polymer, (c) adding sodium tripolyphosphate to the solution of the hyaluronic acid salt, (d) stir-mixing the solutions resulting from steps (b) and (c), spontaneously obtaining the nanoparticles, wherein the active ingredient is dissolved in one of resulting solutions (a), (b) or (c) or in a suspension of the nanoparticles obtained in step (d), to be absorbed in the nanoparticles, wherein the nanoparticles have no covalent bonds between the hyaluronic acid salt, cationic polymer, sodium tripolyphosphate and active ingredient.
>
> **17.** Nanoparticles for the administration of an active ingredient, characterized by a stability of at least one month at ambient temperature, comprising a hyaluronic acid salt, a cationic polymer, sodium tripolyphosphate and the active ingredient as components, without covalent bonds between the components.
>
> **32.** Nanoparticles according to claim 16, storage stable in particle size and zeta potential for at least one month.
>
> **33.** Nanoparticles according to claim 17, storage stable in particle size and zeta potential for at least one month.

Dr. Pena's Declaration then states her awareness that the Application at page 10, lines 23 to page 11, line 3 discloses a process for making the nanoparticles of the aforementioned claims of the Application, as follows:

> "According to a first aspect, the present invention relates to a method of obtaining hyaluronic acid nanoparticles with a diameter less than 1μm, which incorporate an active ingredient, irrespective of the hydrophobic or hydrophilic nature thereof. This method comprises the following steps:
> a)preparing an aqueous solution of a hyaluronic acid salt, preferably in a concentration of between 0.50 and 5 mg/mL;
> b) preparing an aqueous solution of a cationic polymer, preferably in a concentration of between 0.50 and 5 mg/mL;
> c)adding a polyanionic salt to the solution of the hyaluronic acid salt, preferably in a

> concentration of between 0.25 and 1.00 mg/mL;
> d) stir-mixing the solutions resulting from steps b) and c), spontaneously obtaining the nanoparticles"

and that the Application at page 15, lines 9-17 discloses a process for making the nanoparticles of the aforementioned claims, as follows:

> "Example 1
> Hyaluronic acid nanoparticles in the form of sodium salt, chitosan as cationic polymer and sodium triphosphate as crosslinking agent, were prepared according to the previously described method. The hyaluronate and sodium triphosphate solution were added to the chitosan solution, with magnetic stirring, which is maintained for half an hour, permitting the complete evolution of the system towards a stable nanoparticulate form."

Dr. Pena's Declaration states that she conducted the procedure of Example 1 of the Application, and observed that upon addition of hyaluronic acid salt/sodium triphosphate solution to the chitosan solution, and stir mixing of the solutions, nanoparticles were spontaneously formed, without the occurrence of gellation of the mixed solutions.

The Pena Declaration further states that the method of production of nanoparticles by the procedure of the Application was demonstrated not to require an emulsion formation since both solutions, the hyaluronic acid salt/sodium triphosphate solution as well as the chitosan solution, were aqueous solutions.

The Pena Declaration also states that the method of production of nanoparticles by the procedure of the Application was demonstrated not to require dropwise addition of one of solutions, namely, one of the hyaluronic acid salt/sodium triphosphate solution and the chitosan solution, into the other of such solutions.

Dr. Pena states in her Declaration that the method of production of nanoparticles by the procedure of the Application is consistent with an electrostatic interaction between the positively charged cationic polymer (chitosan) and the deprotonated form of hyaluronic acid, and, at the same time, the presence of the polyanionic salt (sodium triphosphate) inducing ionic crosslinking of the cationic polymer, causing the observed spontaneous formation of nanoparticles.

The foregoing provides additional empirical evidence that the claims 16-20 and 29-31 to the nanoparticles of applicants' invention are not incomplete for omitting essential elements, and that such claims are fully proper in form under the provisions of 35 U.S.C. §112, second paragraph.

In response to the §112, second paragraph rejection of claims 29 and 30 for recital of "hyaluronic acid," such claims have now been amended to recite "hyaluronic acid salt" consistent with the terminology of claim 17. It is therefore requested that the §112, second paragraph rejection of such claims, as now amended, be withdrawn.

The Further Empirical Evidence of Stability of the Inventive Nanoparticles

The accompanying 37 CFR 1.132 Declaration of Ana Isabel Vila Pena additionally provides empirical evidence of the stability characteristics that are disclosed in the present application, and now recited in the claims, including recital of at least one month stability in claims 16, 17 (and method claims 1 and 26 subject to rejoinder), and recital of storage stability in particle size and zeta potential in newly added claims 32 and 33.

As set out in the Pena Declaration, Dr. Pena carried out the method of production of nanoparticles by the procedure of the Application, using chitosan as the cationic polymer, and she also carried out a corresponding method of production of nanoparticles in which cationic collagen was used as the cationic polymer in place of chitosan, and a corresponding method of production of nanoparticles in which gelatin was used in place of chitosan.

Her Declaration attests that such nanoparticles were comparatively tested to determine their stability in terms of particle size (nm) and polydispersity index during storage for 4 weeks at 4ºC, with the results shown in the folllowing Table 1, wherein the following abbreviations are used - HA: sodium hyaluronate; COL: cationic collagen; GEL: gelatin; CS: chitosan; P.I: polydispersity index; n.d: not determined.

Table 1. Nanoparticles stability in terms of particle size and polydispersity index during 4 weeks at 4ºC

Formulation	Composition (mass ratio)	Stability at 4ºC									
		0		1 week		2 weeks		3 weeks		4 weeks	
		Size	P.I	Size	P.I	Size	P.I	Size	P.I	Size	P.I
COL/HA	1.2/1	180 ± 4	0.1	238 ± 4	0.3	263 ± 3	0.5	217 ± 6	0.3	198 ± 4	0.3
	1.5/1	202 ± 4	0.1	288 ± 3	0.3	285 ± 6	0.2	237 ± 5	0.2	245 ± 19	0.2
	2/1	217 ± 2	0.1	404 ± 5	0.2	382 ± 15	0.1	427 ± 47	0.4	319 ± 13	0.2
GEL/HA	1/1	266 ± 7	0.1	303 ± 5	0.3	296 ± 6	0.1	n.d	n.d	329 ± 19	0.1
CS/HA	1/2	127 ± 1	0.1	126 ± 1	0.1	134 ± 3	0.1	138 ± 5	0.1	139 ± 2	0.1

HA: sodium hyaluronate; COL: cationic collagen; GEL: gelatin; CS: chitosan; P.I: polydispersity index; n.d: not determined

Her Declaration also attests that such nanoparticles were additionally comparatively tested to determine their stability in terms of particle size (nm) and polydispersity index during storage for 4 weeks at 25ºC, with the results shown in the following Table 2, wherein the following abbreviations are used - HA: sodium hyaluronate; COL: cationic collagen; GEL: gelatin; CS: chitosan; P.I: polydispersity index; n.d: not determined.

Table 2. Nanoparticles stability in terms of particle size and polydispersity index during 4 weeks at 25ºC

Formulation	Composition (mass ratio)	Stability at 25ºC									
		0		1 week		2 weeks		3 weeks		4 weeks	
		Size	P.I	Size	P.I	Size	P.I	Size	P.I	Size	P.I
COL/HA	1.2/1	180 ± 4	0.1	310 ± 13	0.2	282 ± 6	0.4	228 ± 5	0.3	224 ± 7	0.2
	1.5/1	202 ± 4	0.1	397 ± 11	0.3	322 ± 20	0.4	329 ± 29	0.2	289 ± 43	0.4
	2/1	217 ± 2	0.1	480 ± 11	0.1	456 ± 18	0.3	414 ± 13	0.3	353 ± 7	0.3
CS/HA	1/2	127 ± 1	0.1	135 ± 2	0.1	133 ± 1	0.1	146 ± 3	0.1	139 ± 3	0.1

HA: sodium hyaluronate; COL: cationic collagen; GEL: gelatin; CS: chitosan; P.I: polydispersity index

The Pena Declaration attests to the results set out in Tables 1 and 2 above as showing the nanoparticles prepared according to the method of the Application, using different cationic polymers, and subsequently stored at ambient temperatures of 4ºC and 25ºC, to be stable in terms of particle size and polydispersity index over the 4 weeks period of the test.

The Pena Declaration further declares that such nanoparticles were comparatively tested to determine their stability in terms of zeta potential after storage for 4 weeks at 4ºC, and after storage for 4 weeks at 25ºC, with the results shown in the following Table 3, wherein the following abbreviations are used - HA: sodium hyaluronate; COL: cationic collagen; GEL: gelatin; CS: chitosan; and (-): aggregation and/or precipitation.

Table 3. Nanoparticles stability in terms of superficial charge (zeta potential) after 4 weeks at 4 and 25ºC.

Formulation	Composition (mass ratio)	Zeta Potential (mV)	
		4ºC	25ºC
COL/HA	1.2/1	-41 ± 5	-38 ± 5
	1.5/1	-26 ± 7	-39 ± 5
	2/1	-47 ± 6	-50 ± 4
GEL/HA	1/1	-26 ± 5	(-)
CS/HA	1/2	-18 ± 9	-16 ± 8

HA: sodium hyaluronate; COL: cationic collagen; CS: chitosan; GEL: gelatin; (-): aggregation and/or precipitation

The Pena Declaration attests to the results set out in Table 3 above as showing that nanoparticles prepared according to the method of the Application are stable after 4 weeks storage at the ambient temperatures shown in such Table.

Rejection of Claims Under 35 U.S.C.§103 and Traversal Thereof

In the June 10, 2009 Office Action, claims 16-20, 22 and 29-31 were rejected under 35 U.S.C. §103(a) as unpatentable over Prokop U.S. Patent Application Publication 2003/0170313 in view of Calias et al. U.S. Patent Application Publication 2003/0087877.

Such rejection is traversed. (It is noted that claim 22 has been cancelled, thereby rendering the rejection moot in respect of such claim.)

The nanoparticles of applicants' claimed invention differentiate over the Prokop teachings by the presence of hyaluronic acid salt as an ionic component. Hyaluronic acid salt is not mentioned in or derivable from the anionic polymers disclosed in Prokop. There is thus no derivative basis in Prokop for the nanoparticles of applicants' claimed invention, which as a result of the presence of hyaluronic acid salt therein exhibit a surprisingly improved stability.

See in this respect Example 4 of the present application, as well as the accompanying 37 CFR 1.132 Declaration of Ana Isabel Vila Pena providing additional empirical evidence of such surprisingly improved stability.

Such surprisingly improved stability is not derivable from the combination of Prokop in view of Calias, et al., and there is nothing in either of such references that would have caused one of ordinary skill to utilize a hyaluronic acid salt in order to achieve the surprisingly enhanced stability that is evidenced by the nanoparticles of applicants' claimed invention, as demonstrated by Example 4 of the present application and the additional empirical evidence of the 37 CFR 1.132 Declaration of Dr. Pena.

The examiner's attention in this respect is directed to the Manual of Patent Examining Procedure, which in MPEP 2143.01 ("Suggestion or Motivation To Modify the References") states that "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art. *KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, ___, 82 USPQ2d 1385, 1396 (2007)."

Calias, et al. only describe the use of hyaluronic acid as a solid biomaterial over which a therapeutic agent is supported by the formation of covalent bonds (disulfide bridges) between the activated and functionalized carboxyl groups present in hyaluronic acid and thiol groups of the therapeutic agent. There is no indication in Calias, et al. that would lead a person of ordinary skill in the art to assume that hyaluronic acid could be used as an anionic component in a formulation of Prokop in order to enhance the stability of the system.

In contrast to the covalent bonds (disulfide bridges) expressly taught by Calias, et al. between the hyaluronic acid carboxyl groups and the –SH groups of the therapeutic agent, applicants' independent claim 16 recites that "the nanoparticles have no covalent bonds between the hyaluronic acid salt, cationic polymer, polyanionic salt and active ingredient" and independent claim 17 recites "a hyaluronic acid salt, a cationic polymer, a polyanionic salt and the active ingredient as components, without covalent bonds between the components."

Since there is **no derivative basis** in Prokop or Calias, et al. for the applicants' claimed nanoparticles, and, indeed, since Calias, et al. **teach away from applicants' claimed invention by expressly teaching a covalently bond hyaluronic acid component**, and since applicants' claimed nanoparticles exhibit **unexpected and surprisingly improved stability**, applicants' claims 16-20 and 29-31 are patentable over Prokop in view of Calias et al.

Newly added claims 32 and 33, reciting a storage stability in particle size and zeta potential of at least one month, are dependent from claims 16 and 17, respectively, and are patentable over Prokop in view of Calias, et al., for at least the reasons presented above for the patentability of claims 16 and 17.

It is therefore requested that the rejection of claims 16-20 and 29-31 under 35 USC §103(a) be withdrawn, and that such claims 16-20 and 29-31, as well as newly added claims 32 and 33, be allowed.

Request for Rejoinder of Withdrawn Claims 1-15, 21 and 23-28

Consistent with the foregoing remarks establishing the patentability of claims 16-20 and 29-33, it is requested that withdrawn method claims 1-10, 12-15, 21 and 23-28 be rejoined under the provisions of MPEP 821.04, and thereupon be likewise allowed. Such rejoinder is proper since all withdrawn claims 1-10, 12-15, 21 and 23-28, as now amended, embody the limitations of the independent product claims 16 and 17.

Fee Payable for Added Claims 32 and 33

In connection with the cancellation of dependent claims 11 and 22 herein, the addition of new dependent claims 32 and 33 herein entails no net addition of total claims, beyond the number for which payment was previously made. Accordingly, no added claims fee is due.

Request Under 37 CFR 1.136 for Three Months Extension of Time

Request hereby is made under the provisions of 37 CFR 1.136 for a three (3) months extension of the term for reply to the June 10, 2009 Office Action, extending the deadline for response from September 10, 2009 to December 10, 2009.

The fee of $1110 specified in 37 CFR 1.17 (a)(3) for such extension of time is being paid by on-line credit card authorization at the time of EFS filing of this response.

Authorization for Any Additional Fees or Charges

Authorization is also hereby given to charge the amount of any additional fee or charge properly payable in connection with the filing and entry of this response, to Deposit Account No. 08-3284 of Intellectual Property/Technology Law.

CONCLUSION

Based on all of the foregoing, it is requested that all claims 1-10, 12-21 and 23-33 now be allowed.

Respectfully submitted,

/steven j. hultquist/

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The USPTO is hereby authorized to charge any deficiency or credit any overpayment of fees properly payable for this Response, to Deposit Account No. 08-3284 of Intellectual Property/Technology Law.